factors initiate and control the duration and pattern of many annual processes and that such endogenous annual rhythms have significant adaptive value allowing animals to precisely anticipate yearly events such as winter (*19–21*). In temperate lizards, the refractory period commences when temperatures in nature are still sufficiently warm to permit further reproduction. However, the majority of such lizards are oviparous, and their eggs require long underground incubation (on an average of 40 to 50 days). In latitudes having pronounced winters, clutches laid after July and August would produce young when conditions for growth and storage of energy reserves for winter hibernation are less than optimal (*4, 22*). Hence, the frequency of individuals characterized by early breeding and fewer clutches would tend to increase in such populations, eventually adjusting each to coincide with maximum survivorship at any given latitude.

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Chemical Basis for Feeding Adaptation of Pine Sawflies *Neodiprion rugifrons* and *Neodiprion swainei*

Abstract. *Larvae of two pine sawflies,* Neodiprion rugifrons *Midd. and* Neodiprion swainei *Midd., consume only old foliage of jack pine,* Pinus banksiana *Lamb., and leave juvenile foliage intact early in the growing season. The chemical basis for this unique adaptation is a feeding deterrent chemical, 13-keto-8(14)-podocarpen-18-oic acid, which was isolated from juvenile foliage. The content of this deterrent chemical decreases as the foliage begins to mature until needles become acceptable to* Neodiprion swainei *larvae by August (60-day-old foliage) and to second-generation* Neodiprion rugifrons *by September (90-day-old foliage). The precise timing of larval acceptance of juvenile foliage indicates a highly specific relationship between these insects and their host tree based on the composition of chemicals in the foliage.*

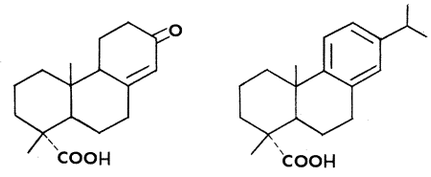
Diprionid sawflies are a well-defined group of nonsocial hymenopterous insects that are closely associated with coniferous forests. They are known by the presence of varieties of distinct races, physiological strains, and species that are well adapted to specific host plants and their physiological conditions (*1*). Such group characteristics make them particularly suitable for studies on the mechanisms of speciation through insect–host plant interactions.

Neodiprion rugifrons Midd. and *Neodiprion swainei* Midd. are species that feed exclusively on jack pine, *Pinus banksiana* Lamb. The most characteristic aspect of their feeding behavior is that in the spring the larvae consume only mature foliage and reject juvenile (current season) foliage. This feeding behavior generally ensures the survival of the host. The surviving foliage then is used for oviposition by the female sawflies and for larval feeding by the next generation late in the growing season.

The purpose of this study was twofold: (i) to reveal the mechanism responsible for differentiation of juvenile foliage from mature foliage by feeding larvae and (ii) to investigate how this mechanism is related to the mode of life of the insects and the host plants.

Preliminary investigations (*2*) indicated that the basis for the sawflies' preferential feeding lies in a feeding deterrent or deterrents present in the juvenile foliage. To test deterrence, a colony of sawfly larvae was transferred to a twig of mature foliage treated on one end with an extract of juvenile foliage and their feeding activities were observed (*3*). Whenever the test extract contained a biologically active deterrent, the larvae did not feed on the treated foliage; instead, they moved away and settled on the portion of the twig that was not treated with the extract.

To isolate and identify the deterrent chemicals, juvenile foliage less than 1 cm long was extracted with *n*-hexane and then with ethyl acetate. The extracts were condensed and the deterrents were separated and isolated by successive chromatography (*4*). Two major deterrent chemicals obtained in pure form were designated compounds A and B.



A B

Judging by the quantities present in the original juvenile foliage and their specific potencies, these compounds account for 64 and 25 percent of the total deterrence, respectively.

The chemical structure of the major feeding deterrent, compound A, was established by rigorous spectroscopic analyses (*5*). To our knowledge, this novel resin acid has not been reported previously. In addition, we synthesized compound A by partial ozonolysis, using neoabietic acid as the starting material (*6*). In laboratory tests, the synthetic compound produced the same degree of deterrence as the natural product. We also demonstrated the effectiveness of compound A in the field. Two isolated young trees were selected in the natural jack pine stands of Arena, Wisconsin. Four or five colonies of second- to third-instar *N. swainei*, consisting of 16 to 43 larvae per colony, were established randomly on old foliage in July 1975. After 24 hours, a 30-ml portion of a solution of compound A (2 mg/ml in 5 ml of ethanol and 25 ml of water) was sprayed over one entire tree. The other tree was sprayed with the same amount of the solvent and water for control. All colonies in the tree treated with compound A were disrupted for at least 3 hours. No larvae commenced feeding for at least 6 hours. After 24 hours approximately half of the larvae were found on the juvenile foliage, an unnatural place for them to settle, and none were observed to feed on this foliage. By contrast, the larvae on

the control tree resumed their normal feeding behavior within 3 hours after the spray was applied.

Compound B was identified as dehydroabietic acid, a known resin acid in pine trees. Examination of the actual concentrations of dehydroabietic acid in jack pine foliage at different periods in its development revealed no appreciable seasonable fluctuation. For instance, the concentration was 0.13 percent in June (juvenile), 0.17 percent in September (90 days old), and 0.06 percent in May (11 months old).

By contrast, we demonstrated that the concentration of compound A decreases as the foliage matures (Fig. 1). We also showed that the timing of larval acceptance of the foliage is closely related to the level of compound A present at the specific stage of growth of the foliage. The *N. rugifrons* larvae in the laboratory bioassay were deterred at a compound A concentration of 0.2 to 0.5 mg/ml (*7*), whereas the *N. swainei* larvae were less sensitive, the corresponding figure being 1 mg/ml. In the field, the latter species begins to accept current-year foliage earlier (in early August) than does the former species (second-generation larvae in mid-September). The compound A concentrations in the foliage in early August and mid-September were 0.025 percent and 0.005 to 0.0125 percent, respectively. Since these values correspond to the concentrations (1 mg/ml and 0.2 to 0.5 mg/ml) of the laboratory test (*7*), the timing of acceptance by each species must be largely controlled by this single chemical.

Our findings basically agree well with the theory advanced by Feeny (*8*) and Rhoades and Cates (*9*) that plants that are easily found ("apparent" or "predictable") tend to develop defensive chemicals that are nontoxic but act in a quantitative manner; that is, the deterring substances are produced in large quantities and work at high concentrations. This theory is mainly based on the observation that later in the season mature oak foliage accumulates defensive substances, tannins, which deter the pest insects' feeding as well as their growth (*10*). However, it is clear that pines are different from oaks in the seasonal sequence of production of the defensive substances. The most likely explanation of this difference is that the juvenile foliage of pines, unlike oak buds, is well exposed in both time and space and is therefore difficult to defend.

It is also interesting that the two sawflies achieved adaptation by using resin acids generally regarded as resistance factors of pine leaves to many organisms

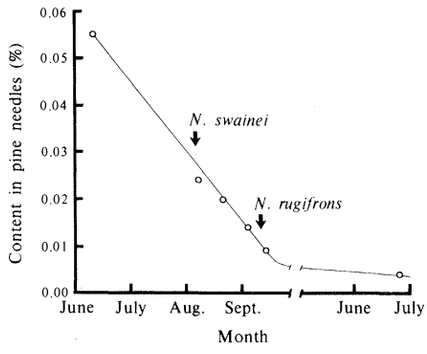


Fig. 1. Seasonal variations of the content of compound A in juvenile jack pine foliage. Arrows indicate the periods when the larvae of the corresponding species begin to accept the juvenile foliage.

(generalists), including insect pests. Sawflies are believed to be among a few groups of organisms (specialists) able to overcome the chemical barrier (*11*). In fact, these chemicals are so noxious to other organisms that they are utilized as defensive chemicals by many diprionid sawflies. For instance, *Neodiprion sertifer* (Geoffr.) is reported to be able to store resin acids, including dehydroabietic acid, in diverticular pouches of the foregut and use them as an effective defensive excretion against its predators (*12*).

Despite the evolution of the ability of sawflies as a group to "crash" through the chemical defense of the coniferous host tree, a few resinous chemicals remain deterrent to some species. Since nine species of *Neodiprion* sawflies are known to show at least a preferential discrimination against new growing needles, such a trend is not confined to the two species we investigated. Whether such an adaptation represents counter-evolution of the host tree or incomplete evolution of the sawflies is an interesting question.

Close coevolution of herbivorous insects and host plants is known to be more pronounced in cases involving specialists, particularly monophagous species, which are limited in their mode of life and geographical distribution by those aspects of the host plant (*9*). The "apparent" plants that have already developed quantitative defense mechanisms against generalists may be expected to receive pressures from specialists such as sawflies. Thus, further evolution of specialized defenses becomes necessary.

Modification of some resin acids to develop a specialized deterrent appears to be a natural course of such counter-evolution on the part of the host tree. Moreover, if one assumes that plant defenses are costly in the time and energy budget of plants (*8*), it is clearly advantageous to utilize resin acids for the second line of defense through a minor modification with a limited distribution.

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3. To monitor the presence of chemical deterrents we devised the following laboratory bioassay. A 7- to 10-cm twig with mature foliage was rinsed with distilled water and dried, and then separated into two parts by removing the foliage of the center portion. Ten pairs of pine needles were left at each end. Needles at one end were treated with a solvent extract from juvenile foliage and those at the other end were treated with the solvent. The twig was air-dried at room temperature for more than 30 minutes and placed in a covered plastic container (6 by 7 by 21 cm) the bottom surface of which was covered with a green paper. Ten third- to fifth-instar larvae were placed on the needles at each end, and their feeding behavior was observed until most of the larvae (> 80 percent) started feeding on needles at one or both ends. This usually required 3 to 4 hours. The number of larvae on needles on each end was recorded at least every 30 minutes. Whenever more than 70 percent of the larvae moved to and settled on the untreated needles, a positive score for feeding deterrence was recorded.
4. To isolate the deterrent components, 2.88 kg of juvenile jack pine foliage less than 1 cm long was extracted three times with methanol (three 18-liter portions), and the solvent was evaporated. The residue was extracted with *n*-hexane (three 1.7-liter portions) and then ethyl acetate (three 1.7-liter portions). Both *n*-hexane and ethyl acetate showed biological activity, but not the residues. The solvents were removed in vacuo and both fractions were chromatographed on a silicic acid (450 g) column. The major deterrent, compound A, was eluted with a mixture of benzene and ethyl acetate (4:1), and compound B was eluted with a 10:1 mixture of the same solvents. The thin-layer chromatography (TLC) conditions employed to purify these two compounds were silica gel HF_{254} with benzene and acetone (2:1) for A and *n*-hexane and ether (1:1) for B as mobile phases. The R_F positions in the TLC systems were 0.35 for A and 0.40 for B. The details of the isolation and identification procedures for these compounds will be published elsewhere (*13*).
5. The main spectroscopic data for the methyl ester of compound A are: mass spectroscopic fragments at mass-to-charge (*m/e*) ratios 290 (M^+), 259, and 231; ultraviolet absorption spectrum in ethanol at 240 nm (extinction coefficient, 8.3×10^3); infrared spectral peaks at 1735 and 1680 cm^{-1}; and proton magnetic resonance spectrum in $CDCl_3$ with peaks at 0.849 [3 H, singlet (S)], 1.231 (3 H, S), 3.681 (3 H, S), and 5.870 (1 H, S) parts per million.
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Visual Sensitivity: Significant Within-Species Variations in a Nonhuman Primate

Abstract. *Among squirrel monkeys (*Saimiri sciureus*) there are significant sex-related differences in visual sensitivity. As measured behaviorally in an increment-threshold task, a sample of males was found to be substantially less sensitive to long-wavelength (640-nanometer) light than a group of females tested in the same way, although the two groups showed no significant differences in sensitivity to a middle-wavelength (540-nanometer) light. The two groups also differed on a test designed to measure the effects of chromatic adaptation.*

The presence of significant variations in sensory capacity among human populations has been documented for a number of sensory dimensions. Particularly well known, and by far the most extensively studied, are those variations collectively categorized as defective color vision (*1*). The differences in visual capacity between so-called color-defective observers and those with normal color vision can be substantial. For example, sensitivity to long test wavelengths may be on the order of 1 log unit or more higher in individuals with normal color vision than in those having one of the most common types of color-defective vision, protanopia (*2*). Study of these naturally occurring differences has occupied a prominent position in vision research, primarily because of the possibility of obtaining useful inferences about biological mechanisms through a comparison of normal and defective systems. However, because it is difficult to directly test hypotheses about mechanisms in human subjects, it would be useful if significant within-species variations in visual capacity could be identified in a nonhuman species. I have recently found such differences in the South American squirrel monkey and now report the results of behavioral tests of members of this species to indicate the nature and the magnitude of their variation in visual capacity (*3*). One of the interesting aspects of this variation is that it appears to be linked to gender.

All behavioral tests were conducted in the context of a forced-choice discrimination task. Three stimulus panels were transilluminated by light sources located outside a small test chamber. Each of these circular panels subtended 18° to 35° of visual angle, depending on the position of the unrestrained monkey. The interior of this chamber was continuously illuminated with an achromatic light (mean illuminance of 60 lux). Animals were deprived of food for 22 hours before each daily test session. Through an operant shaping procedure they were initially taught to receive a food reinforcement—a 97-mg banana-flavored pellet—by pressing a lever mounted adjacent to that stimulus panel which was differently illuminated from the other two. Between test trials the three panels were identically illuminated. A test trial consisted of opening a shutter which added light to one of the three panels. A cueing tone was used to demark the occurrence of this observation interval. A noncorrection procedure was used. The position of the positive panel (that is, the panel to which light was added) was random within the constraint that all three panels were positive an equal number of times in each test session.

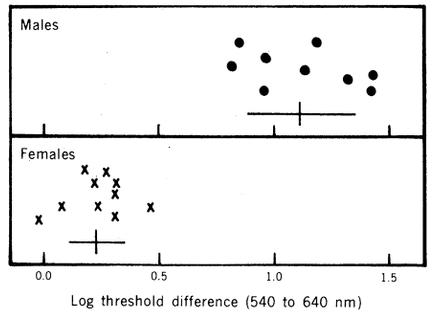

Fig. 1. Visual sensitivity of squirrel monkeys as measured in an increment-threshold task. The values plotted represent differences between the thresholds for 540- and 640-nm test lights. Each symbol represents one animal. These symbols are positioned arbitrarily on the ordinate for ease of viewing. The vertical line indicates the mean difference for each group, and the horizontal line encloses two standard deviations.

The subjects were adult squirrel monkeys (*Saimiri sciureus*). All were feral animals of the "Roman Arch" phenotype (*4*), but they were otherwise unselected. The first test consisted of measuring the monkeys' sensitivity to two different monochromatic lights, 540 and 640 nm (half-energy bandwidths of 10 nm). Either of the stimuli at a high-intensity level was added to one of the three panels, all of which were continuously illuminated with achromatic light (luminance = 3 cd/m^2, color temperature = 5820°K). During the initial training period, the two test lights were presented an equal number of times in each test session. Once the animal acquired the discriminations (as indicated by performing at, or close to, 100 percent correct), the intensity of each monochromatic light was decreased in steps of 0.3 log unit until a level was reached that produced chance discrimination (33 percent correct). At this point the full range of intensities for each test wave length was presented until, over test sessions, a total of 50 trials had been accumulated at each wavelength-intensity combination. From these results the intensity of the light required to produce threshold-level discrimination (arbitrarily defined as 50 percent correct) was determined. Thresholds for each test wavelength were determined twice, and if any improvement occurred between the first and second determinations, a third set of measurements was also made.

The results from tests of 19 monkeys (nine males, ten females) are shown in Fig. 1. In order to minimize the effects of individual differences in response criterion, the data plotted are the differences in threshold for the 540- and 640-nm test lights. The male monkeys show a much greater difference in sensitivity to the 540- and 640-nm lights than the females do. The mean threshold difference between these two wavelengths for the females is 0.23 log unit, whereas for the males, the corresponding comparison is 1.12 log units ($t = 4.56$, $P < .001$). There is in this sample no overlap between the two groups; that is, the most sensitive male was less sensitive to the long test wavelength than the least sensitive female. On the other hand, the two groups showed no significant differences in threshold to the 540-nm test wavelength ($t = 0.79$, $P > .05$).

It seemed unlikely that the large differences shown in Fig. 1 could be due to any systematic differences in response criteria or to some sort of preretinal filter present in some, but not all, animals. Nevertheless, these possibilities were evaluated by redetermining thresholds